Molecular Therapies for Vascular Diseases

Gary H. Gibbons and Victor J. Dzau*

Vascular disease is the most common cause of death in the industrialized world. Although significant progress has been made in treating these disorders, more therapeutic agents must be developed that effectively prevent, arrest, or reverse this disease. Recent insights into the pathogenesis of vascular disease have opened up a new frontier of molecular therapies that target molecules as diverse as adhesion molecules and transcription factors. The biological rationale for these new therapies and their prospects for success are discussed.

Insights into the pathogenesis of vascular disease must be based on an understanding of the regulatory processes that maintain the structure and homeostatic functions of the healthy vessel. The vessel wall is normally composed of an endothelial cell lining that is tightly juxtaposed to a medial layer of vascular smooth muscle cells and enwrapped by an adventitial layer of connective tissue. The endothelial cell lining is ideally situated at the interface between the blood and the vessel wall to serve as a sensor and transducer of signals within the microenvironment. Endothelial cells orchestrate the homeostatic balance of the vessel through the production of factors regulating vessel tone, coagulation state, cell growth, cell death, and leukocyte trafficking. Vascular smooth muscle cells maintain the contractile tone of the blood vessel in response to vasoactive substances and release cytokines and other growth regulatory factors. Together with fibroblasts these cells also elaborate extracellular matrix proteins as well as proteases that determine vessel structure. Occlusive vascular disease is characterized by the abnormal accumulation of vascular smooth muscle cells, inflammatory cells, and extracellular matrix proteins within the intimal space between the endothelial lining and the medial layer (neointima formation) (*1*). The therapeutic challenge is to preserve normal vessel structure by developing agents that prevent, arrest, or reverse this process of neointima formation.

Atherosclerosis is the most common form of occlusive vascular disease and will be the focus of this review. The pathogenesis of atherosclerosis involves a series of critical events that include endothelial dysfunction, infiltration of inflammatory cells into the vessel wall, alterations in vascular cell phenotype, and vascular remodeling (*1*). Current therapies are directed either at reducing the risk factors that promote atherosclerosis (for example, cholesterol) or enhancing blood flow by interventions such as balloon angioplasty or surgical revascularization. As the molecular bases of these pathogenic events become elucidated, they may provide opportunities for the development of new molecular therapies that can modify the course of vascular disease (Fig. 1).

The authors are at the Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, CA 94305-5246, USA.

*To whom correspondence should be addressed.

Endothelial Cell Dysfunction

The development of endothelial cell dysfunction is characterized by an impairment in vasorelaxation and increased adhesiveness of the endothelial cell lining. This alteration in endothelial function is one of the harbingers of vascular disease and is manifest in a wide spectrum of vascular disorders including hypertension, transp coronary vascular disease and atherosc sis. The dysfunctional endothelium pr poses vascular tissue to vasoconstric platelet-thrombus formation, and inflan tory cell infiltration into a plaque. The ical sequelae of atherosclerotic vascula ease such as a myocardial infarction ap to involve a cascade of events initiate inflammatory cell adhesion to the end lium, leukocyte infiltration into the pl protease-mediated weakening of the p structure, plaque rupture, thrombosis, eventually tissue ischemia. Thus, cha in the endothelial cell's function as a keeper governing leukocyte traffic can influence the natural history of v lar disease.

Several lines of evidence indicate endothelial cell dysfunction is assoc with alterations in the cell redox Many of the risk factors associated atherosclerotic vascular disease, suc hyperlipidemia, diabetes, and hype sion, promote an oxidative stress. In hyperlipidemia increases the generat superoxide anions and thereby pro the oxidation of low density lipop (LDL) cholesterol within the vessel Similarly, the potentiated atherog observed in diabetics may be related induction of oxidative stress by adv glycation end products. Vasoactive f that promote hypertension, such as tensin II, also induce the generati reactive oxygen species. These ob tions raise the possibility that diffe in the vascular response to oxidative may contribute to the genetic susce ity to atherosclerosis (*2*).

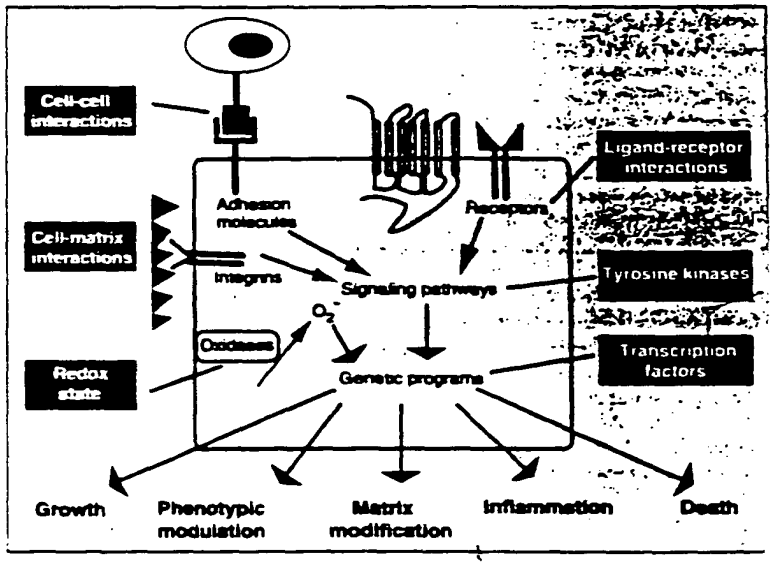

Fig. 1. Schematic model of a "generic" vascular cell, showing the potential targets for t therapeutics in vascular disease.

The alteration in endothelial cell redox state is accompanied by increased adhesive interactions between the endothelium and inflammatory cells. Mechanistically, this change may occur because reactive oxygen species can function as signaling molecules that mediate increased activity of the transcription factor nuclear factor kappa B (NF-κB). Increased activity of NF-κB induces a coordinated up-regulation in the expression of adhesion molecules such as vascular cell adhesion molecule–1 (VCAM-1) and chemokines such as monocyte chemoattractant protein–1 (MCP-1) (3).

The impairment of vasorelaxation reflects the enhanced catabolism of nitric oxide (NO) caused by the increased generation of reactive oxygen species. In addition to its role as an endothelium-derived vasodilator, NO appears to be an endogenous inhibitor of vascular smooth muscle cell growth and migration. NF-κB activity, and the expression of pro-inflammatory molecules such as VCAM-1 and MCP-1. Hence, the two characteristic features of endothelial dysfunction—impaired vasorelaxation and increased adhesiveness—appear to result in part from a decrease in NO bioactivity. This relative deficiency in NO activity predisposes vascular tissue to atherosclerotic lesion formation (4). Thus, the alteration in endothelial cell redox state provides a molecular link between hyperlipidemia, endothelial dysfunction, and atherosclerotic lesion formation. On the basis of these observations, therapeutic strategies that modulate the endothelial cell redox state are postulated to help reverse endothelial dysfunction and thereby prevent the progression of vascular diseases such as atherosclerosis.

Dysregulated Cell-Cell and Cell-Matrix Interactions

Under normal circumstances the endothelium is a nonadhesive surface that actively inhibits cell-cell interactions with blood elements such as platelets or leukocytes. The normal structure of the vessel is maintained by integrin-mediated interactions between vascular cells and the surrounding extracellular matrix. The pathogenesis of vascular diseases is characterized by perturbations in cell-cell and cell-matrix interactions that predispose vascular tissue to thrombosis, inflammation, and atherosclerotic lesion formation.

The natural history of atherosclerotic vascular disease is punctuated by acute morbid events involving tissue ischemia. Acute ischemic syndromes such as myocardial infarction or unstable angina are incited by a fracture or rupture of the atherosclerotic plaque. A thrombus forms at the site of plaque rupture as a result of increased adhesion of platelets to the vessel wall, activation of thrombin, and fibrin deposition. New selective inhibitors of various components of the coagulation cascade, including thrombin antagonists, have been created and are currently being tested in clinical trials. In addition, a new class of antithrombotic agents such as IIb/IIIa inhibitors has been developed based on an understanding of the role of specific integrins as molecular determinants of platelet adhesion and aggregation (5). This strategy of inhibiting cell-cell interactions provides a promising therapeutic approach to the prevention of vascular complications such as myocardial infarction.

In addition to therapeutic strategies that focus on altering endothelial cell redox state or enhancing NO activity, an alternative strategy may involve direct antagonists of leukocyte-endothelial interactions. The regulation of this inflammatory response by the endothelium involves a complex interplay between adhesion molecules expressed on the cell surface that govern cell-cell interactions as well as production of chemoattractants, and cytokines produced by both leukocytes and endothelial cells (6). The influx of inflammatory cells into the vessel wall is a multistep process involving sequential interactions between adhesion molecules from the selectin family (for example, P-selectin), chemokines (for example, MCP-1), adhesion molecules from the integrin family (for example, VCAM-1) as well as chemoattractants [for example, platelet activating factor (PAF)], and cytokines (for example, interleukin-1).

In principle, therapies targeted at different points in the leukocyte trafficking process may reduce the inflammatory response involved in vascular lesion progression or the initiation of acute ischemic events (or both). One strategy would be to block the key cytokines that potentiate the inflammatory response, such as tumor necrosis factor (TNF) or certain interleukins. Such a blockade has been achieved in animal models through use of soluble receptors. Alternatively, studies in animal models and in vitro model systems suggest that selective ligand-receptor antagonists directed against leukocyte adhesion molecules such as P-selectin and intercellular adhesion molecule–1 (ICAM-1), or paracrine factors such as PAF, may also be effective in reducing the inflammatory response common to many forms of vascular disease (7).

A growing body of evidence indicates that the inflammatory response involves the activation of an endothelial cell genetic program that promotes the coordinate up-regulation of adhesion molecules and cytokines. In particular, the transcription factors NF-κB and Egr-1 appear to play centr roles in this inflammatory response (3, 5 Selective inhibitors targeted against tra scription factors that orchestrate the ge activation events necessary for leukocy adhesion and trafficking may prove vai able as vascular therapeutics.

Integrins are important targets for ther peutic interventions because vascular c behavior is determined by both cell-cell a cell-matrix interactions. Each of the cellu processes involved in neointima formatic appears to be modulated by signals deriv from integrin-substrate interactions. The i tegrin $\alpha_v\beta3$, for example, is required bo for prevention of endothelial cell apopto and for the cell-matrix interactions nece sary for vascular smooth muscle cell migr tion. Although therapeutic strategies d signed to inhibit cell migration by prever ing matrix degradation have equivocal effe tiveness (9), blockade of $\alpha_v\beta3$-mediat interactions has been shown to attentua lesion formation after vascular injury in a imal models (*10*). Moreover, it is speculat that the reduction in morbid events (i example, recurrent ischemia) in patients u dergoing angioplasty after the administr tion of the 7E3 antibody (an antagonist the IIb/IIIa integrin) (*11*) may actually related to the capacity of this antibody block $\alpha_v\beta3$ as well as IIb/IIIa. Overall, the studies suggest that cell adhesion molecu represent a new class worthy of a drug d velopment program for vascular diseases.

Dysregulated Cell Growth and Cell Death

A fundamental pathological feature of v cular disease is marked by the abnorm accumulation of cells within the intir space, resulting in neointimal lesion form tion produced by alterations in the homo static balance between cell growth and c death. Studies in experimental animal mo els have identified several growth factors (example, platelet-derived growth fac (PDGF), transforming growth factor–β (TGFβ1), basic fibroblast growth fac (bFGF), and angiotensin II (Ang II)] th may play important pathogenic roles. (However, most animal models studied date may not accurately simulate the co plex process of lesion formation in hum vascular disease. Clinical trials based on t geted blockade of a single growth fac have failed to recapitulate the efficacy d umented in animal models (*12*). Given multiplicity of the growth factors invol in neointima formation and the comple of the disease process in the clinical cont it is unlikely that drugs targeted at sin growth factors or their receptors will pr to be effective vehicles of vascular thera A more successful approach may be to tar

components of the intracellular signaling cascades that are shared by many growth regulatory molecules.

PDGF, TGF-β1, and Ang II are important mediators of vascular lesion formation, yet represent different classes of receptor molecules. Despite their differences, they show substantial overlap in signal-transduction mechanisms. For example, Ang II, which interacts with a specific heterotrimeric GTPase-binding protein (G protein)–coupled receptor, can also activate various components of the tyrosine kinase signaling cascade. This cross talk may be related to ligand-independent transactivation of tyrosine kinase receptors in response to stimulation of G protein–coupled receptors (*13*). Although selective inhibitors of these common signaling pathways may serve as effective blockers of vascular lesion formation, one caveat to this approach is the potential for toxicity given the broad array of cellular processes governed by tyrosine kinases. Local delivery of these agents into the vascular lesion may be necessary to avoid systemic toxicity.

Regulation of the intimal cell population requires a delicate balance between cell influx, cell growth, and cell death. Indeed, recent studies suggest that a decrease in apoptosis may be a prominent feature of vascular lesion formation (*14*). Many of the molecules previously identified as growth factors also appear to function as factors that prevent vascular cell death. For example, the angiogenic agent bFGF promotes endothelial cell survival in addition to inducing endothelial cell mitogenesis. Similarly, PDGF and Ang II promote vascular smooth muscle cell survival as well as stimulate cell growth (*15*). The cellular signaling pathways that mediate this anti-apoptotic response remain to be further characterized. Recent studies with neuronal, hematopoietic, and endothelial cells indicate that mitogen-activated protein kinase (MAPK) and phosphoinositol 3-kinase may mediate anti-apoptotic signals, whereas the JNK kinases may be pro-apoptotic (*16*). Changes in redox state may also modulate the activation of cell death programs (*17*). Integrins, in particular $\alpha_v\beta_3$, also appear to be important determinants of vascular cell survival (*18*). All of these signaling pathways appear to converge on a cell death machinery governed by the relative expression of the Bax-Bcl-2 family and the ICE (interleukin converting enzyme)–like cysteine proteases. Therapeutic strategies that either up-regulate the expression of pro-apoptotic factors or down-regulate the expression of anti-apoptotic factors may represent an exciting opportunity for inhibition of vascular lesion formation.

Modulation of Vascular Cell Phenotype

Several lines of evidence indicate that the vascular smooth muscle cells within the neointima constitute a distinct cell population with altered phenotypic characteristics. Intimal smooth muscle cells exhibit altered expression of transcription factors linked to myogenic differentiation, growth factors, apoptosis regulatory genes, integrins, matrix proteins, and matrix proteases (*1*). The net result of these changes in cell properties is a population of smooth muscle cells that have an increased propensity to proliferate, migrate, and elaborate a more abundant extracellular matrix. The molecular basis of this alteration in intimal smooth muscle cell phenotype remains to be clarified, but may reflect the activation of a genetic program that recapitulates the cell properties observed during fetal development (*1*). For example, the homeobox gene *Gax*, which is expressed in differentiated medial smooth muscle cells, becomes down-regulated in the context of vascular injury. Transfer of the *Gax* gene into vascular smooth muscle cells after balloon injury has been shown to inhibit cell proliferation and neointima formation in vivo (*18*). Conversely, MEF-2, a set of candidate transcription factors that regulate muscle-specific genes, is selectively up-regulated after vascular injury and thus may play a role in the phenotypic modulation of intimal cells (*19*). Agents that regulate the activity of these transcription factors merit consideration as vascular therapeutics.

Restenosis as a Paradigm for Molecular Therapy

One of the principal treatments for occlusive vascular disease is angioplasty, a procedure in which a balloon is inserted into the vessel and then inflated to dilate the area of narrowing. In 30 to 50% of cases, the initial increase in lumen dimensions is followed by a re-narrowing (restenosis) of the vessel over a time course of 3 to 6 months. This process of restenosis is complex and results from cellular hyperplasia within the neointima, the organization of thrombus within the vessel wall, and a process of vascular remodeling or shrinkage in the overall vessel dimensions. There is no clinically effective therapy for this disease.

Several molecular therapies have already been designed and tested in animal models. Angioplasty denudes the vessel of endothelial cells that would normally generate paracrine inhibitors of vascular smooth muscle migration and proliferation. Thus, one approach has been directed at replacing a key product of endothelial cells, NO synthase. The endothelial cell–type NO synthase gene was transfected locally into the vessel wall after balloon injury in a rat model of neointima formation. The resultant local generation of NO substantially inhibited the cell proliferation, migration, and matrix production required for neointima formation (*20*). Indeed, preliminary results of clinical studies involving systemic administration of NO-donor drugs confirm the utility of augmenting NO activity as a means of preventing restenosis (*21*).

The abnormal cell proliferation that characterizes restenosis is driven by a multiplicity of growth factors. One therapeutic approach that overcomes this problem is cytotoxic therapy. Local transfection of the herpes simplex virus thymidine kinase (HSV-TK) gene, together with systemic administration of the prodrug ganciclovir, was successful in inhibiting neointima formation in a porcine model (*22*). Still another strategy that avoids the tissue damage induced by cytotoxic therapy is cytostatic therapy, which is aimed at the inhibition of cell cycle progression. Local delivery of antisense oligonucleotides directed against the expression of cell cycle regulatory genes such as proliferating cell nuclear antigen (PCNA), Cdc2, c-Myb, and c-Myc inhibited neointima formation in several models of vascular lesion formation (*23*). The coordinated induction of these cell cycle regulatory genes is mediated by the transcriptic factor E2F. Oligonucleotides containing the E2F cis element sequence can function as "decoys" that bind selectively to E2F within the cell. Intracellular delivery of the E2 transcription decoys results in the prevention of E2F-mediated up-regulation of cell cycle regulatory genes. Transfection of E2 transcription factor decoy oligonucleotides into the vessel wall inhibits neointimal formation formation in vivo (*24*). Similar responses have been observed with a gene augmentation approach in which cell cycle inhibitors such as p21, dominant-negative R mutants, or mutant retinoblastoma (R gene are overexpressed locally in vascular cells to inhibit cell proliferation after vascular injury in various animal models of neointima formation (*25*). Consistent with the concept of cytostatic therapy, systemic administration of conventional pharmacological agents such as rapamycin that inhibit cell cycle kinases also inhibits vascular lesion formation in rat and porcine models (*2*). Interestingly, the induction of cell cycle arrest has additional therapeutic consequences. For example, inhibition of cell cycle regulatory genes also appears to inhibit cell migration and matrix production (*27*) and can also trigger the activation of vascular cell apoptosis (*28*). The successful use of local radiation therapy to inhibit neointima formation after vascular injury may reflect a similar combination of cell cycle arrest

vascular cell apoptosis (29).

Based on our current state of knowledge, the most effective therapy for occlusive vascular disease will likely combine intravascular stenting (a cylindrical metal strut that expands the lumen) with an antiproliferative therapy. Adjunctive therapeutic strategies designed to target adhesion molecules that function in platelet-thrombus formation and cell-matrix interactions also show promise (11).

Vein Bypass Graft Failure

Bypass surgery with vein grafts is the standard surgical approach to treat occlusive vascular diseases. Vein grafts are conduits that restore normal tissue blood flow by circumventing the occlusive arterial lesion. However, about 50% of the grafts occlude within 5 to 10 years because of neointimal hyperplasia and accelerated atherosclerosis within the graft. In principle, a genetic engineering strategy might allow the creation of venous grafts adapted to the arterial circulation yet resistant to neointima lesion formation. Experiments with a rabbit model showed that transfection of antisense oligonucleotides directed against the expression of the cell cycle regulatory genes PCNA and Cdc2 inhibited DNA synthesis and neointima formation within the vein graft. This strategy prevented lesion formation, yet allowed the adaptive thickening of the vein wall necessary to withstand the mechanical stress of the arterial circulation. The genetically engineered grafts were resistant to atherosclerosis when implanted in rabbits with severe hyperlipidemia as compared with the accelerated atherosclerosis observed in control grafts (30).

This example illustrates the feasibility of applying genetic engineering technology to vascular bypass grafts. Vascular grafts can be used as carrier systems for the implantation of cells that are genetically engineered ex vivo to secrete paracrine factors with desired activities (for example, anti-thrombotic agents). Alternatively, the vein graft cells can be transfected in situ with expression vectors carrying genes encoding vasodilators, anti-inflammatory molecules, or angiogenic factors (31). One advantage of this approach is that it allows manipulation of the grafts ex vivo, optimization of transfection efficiency, and minimization of toxicity. Such "designer vessels" may one day have important clinical applications.

Future Directions and Challenges in Vascular Therapeutics

Based on our present understanding of the molecular basis of vascular disease, there are a wealth of potential therapeutic opportunities (Table 1). However, most molecular therapies described here have focused on short-term interventions in acute processes, whereas the pathogenesis of atherosclerosis is characteristically a slow process. Thus, the ideal strategy is to interrupt the early steps of atherosclerotic lesion formation by developing long-term therapies directed at the molecules that are important initiators in the pathogenesis of vascular disease.

The development of new cardiovascular therapies will be facilitated by the rapid progress being made in rational drug design, the use of high-throughput combinatorial libraries as an efficient means of screening candidate drugs, and the development of animal models of vascular disease that reveal critical pathogenic molecules for drug targeting. The advent of transgenic mouse, rat, and rabbit models of vascular disease will facilitate this process.

The advancement of molecular therapeutics for vascular disease faces several major challenges that will determine its success. For example, as new drugs are discovered, target specificity and selectivity will be a recurrent issue. Local delivery of drugs within the vasculature to circumvent problems of systemic toxicity will require further study, and implantation of intravascular drug delivery devices to enhance the bioavailability of new classes of drugs such as nucleic acids and recombinant proteins (32) remains to be demonstrated. Moreover, further advances in drug delivery technology are necessary to overcome the inherent limitations of this approach in the treatment of chronic processes such as atherosclerosis. Another challenge is the selection of animal models for drug evaluation. The question whether efficacy documented in experimental animal models can predict clinical effectiveness in humans continues to be a vexing problem.

Finally, one of the most difficult issues is the establishment of criteria for evaluating the clinical efficacy of molecular therapies that influence slowly progressive pathological processes such as atherosclerosis. For example, it is conceivable that therapeutic agents that modify biological processes within the vasculature may have beneficial clinical effects without inducing significant changes in vessel structure as assessed by conventional clinical techniques. It is not clear whether measurements of vascular morphology (by angiography and intravascular ultrasound) are appropriate end points for defining the clinical efficacy of molecular therapies. Can we define the clinical utility of a new drug based on its effect on a target molecule activity such as cell adhesion or redox state? Will these surrogate markers of clinical efficacy be an acceptable alternative to assessing changes in vessel structure or the incidence of clinical events such as myocardial infarction? Are extensive clinical trials designed to demonstrate reductions in clinical events (for example, myocardial infarction or mortality) a prerequisite for the approved use of new vascular therapeutic agents? These are only a few of the challenges that must be surmounted for the successful development of molecular therapies for vascular disease.

Table 1. Molecular therapies for vascular diseases: pathologic bases and potential therapeutic targets.

Pathologic event	Therapeutic target
Endothelial dysfunction	NO inducer or donor; antioxidants
Endothelial injury	VEGF; FGF
Cell activation and phenotypic modulation	MEF-2 and Gax modulators; NF-κB antagonists; cell cycle inhibitors
Dysregulated cell growth	E2F decoys; RB mutants; cell cycle inhibitors
Dysregulated apoptosis	Bax or CPP32 inducers; Bcl-2 inhibitors; integrin antagonists
Thrombosis	IIb/IIIa blockers; tissue factor inhibitors; anti-thrombin agents
Plaque rupture	Metalloproteinase inhibitors; leukocyte adhesion blockers
Abnormal cell migration	Integrin antagonists; PDGF blockers; plasminogen activator inhibitors
Matrix modification	Metalloproteinase inhibitors, plasmin antagonists; matrix protein cross-linking modifiers

REFERENCES AND NOTES

1. R. Ross, *Annu. Rev. Physiol.* 57, 791 (1995); Schwartz, D. deBlois, E. R. O'Brien, *Circ. Res.* 445 (1995); G. H. Gibbons and V. J. Dzau, *N. Engl. Med.* 330, 1431 (1994).
2. R. W. Alexander, *Hypertension* 25, 155 (1995); Steinberg, *Adv. Exp. Med. Biol.* 369, 39 (1995); Ohara *et al.*, *Circulation* 92, 898 (1995); Schmidt *et al.*, *J. Clin. Invest.* 96, 1395 (1995); Gruendling *et al.*, *Circ. Res.* 74, 1141 (1994); F *et al.*, *J. Clin. Invest.* 94, 877 (1994).
3. N. Maru *et al.*, *J. Clin. Invest.* 92, 1866 (199 Collins *et al.*, *FASEB J.* 9, 899 (1995).
4. H. B. Peng, P. Libby, J. K. Liao, *J. Biol. Chem.* 14214 (1995); R. De Caterina *et al.*, *J. Clin. I* 96, 60 (1995); A. J. Cayatte, J. J. Palacio Horten, R. A. Cohen, *Arterioscler. Thromb.* 1 (1994); J. P. Cooke and P. S. Tsao, *ibid.*, p. 6
5. B. S. Coller, *Circulation* 92, 2373 (1995).
6. T. A. Springer, *Annu. Rev. Physiol.* 57, 827 (1
7. A. Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. U.S.* 10535 (1991); L. P. Avila *et al.*, *ibid.* 92, (1995); M. Isobe, H. Yagita, K. Okumura, A. *Science* 255, 1125 (1992); A. S. Weyrich *et al.*, *Invest.* 91, 2620 (1993); A. S. Weyrich *et al.*, *ib* 2297 (1995); J. M. Heasy *et al.*, *ibid.* 96, 2322 (
8. L. M. Khachigian, V. Lindner, A. J. Williams, T ins, *Science* 271, 1427 (1996).
9. N. Zempo *et al.*, *Arterioscler. Thromb. Vasc. Bi* 28 (1996).

10. P. C. Brooks *et al.*, *Cell* 79, 1157 (1994); L. Liaw *et al.*, *J. Clin. Invest.* 95, 713 (1995); H. Matsuno, J. M. Stassen, J. Vermylen, H. Deckmyn, *Circulation* 90, 2203 (1994).
11. E. J. Topol *et al.*, *Lancet* 343, 881 (1994).
12. D. P. Faxon *et al.*, *J. Am. Coll. Cardiol.* 25, 362 (1995).
13. D. A. Linseman, C. W. Benjamin, D. A. Jones, *J. Biol. Chem.* 270, 12563 (1995); M. B. Marrero *et al.*, *ibid.*, p. 15734; M. B. Marrero *et al.*, *Nature* 375, 247 (1995); I. E. Zohn *et al.*, *Mol. Cell Biol.* 15, 6160 (1995); H. Daub, F. U. Weiss, C. Wallasch, A. Ullrich, *Nature* 379, 557 (1996).
14. D. K. M. Han *et al.*, *Am. J. Pathol.* 147, 267 (1995); Y. J. Geng and P. Libby, *ibid.*, p. 251; S. Kondo *et al.*, *Exp. Cell Res.* 213, 428 (1994).
15. M. R. Bennett, G. I. Evan, S. M. Schwartz, *J. Clin. Invest.* 95, 2266 (1995); M. Polman, T. Yamada, M. Horiuchi, G. H. Gibbons, *FASEB J.* 9, A351 (1995).
16. Z. Xia, M. Dickens, J. Raingeaud, R. J. Davis, M. E. Greenberg, *Science* 270, 1326 (1995); R. Yao and G. M. Cooper, *ibid.* 267, 2003 (1995); M. Varnai *et al.*, *Nature* 380, 75 (1996).
17. M. Sundaresan, Z.-X. Yu, V. J. Ferrans, K. Irani, T. Finkel, *Science* 270, 296 (1995); J. C. Tsai *et al.*, *J. Biol. Chem.* 271, 3667 (1996).
18. L. Wei *et al.*, *J. Biol. Chem.* 270, 5457 (1995); K. Walsh *et al.*, abstract presented at Keystone Symposium, Keystone, CO, 29 January 1996.
19. A. B. Firulli *et al.*, *Circ. Res.* 78, 196 (1996); V. Andres, S. Fisher, P. Weinsch, K. Walsh, *Mol. Cell Biol.* 15, 4272 (1995).
20. H. E. von der Leyen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92, 1137 (1995).
21. J. J. Ferguson, *Circulation* 90, 4 (1994).
22. T. Ohno *et al.*, *Science* 265, 781 (1994).
23. M. Simons *et al.*, *Nature* 359, 67 (1992); R. Morishita *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90, 8474 (1993); Y. Shi *et al.*, *Circulation* 90, 944 (1994).
24. R. Morishita *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92, 5855 (1995).
25. M. W. Chang *et al.*, *Science* 1995 267, 518 (1995); M. W. Chang *et al.*, *J. Clin. Invest.* 96, 2260 (1995); C. Indolfi *et al.*, *Nature Med.* 1, 541 (1995).
26. C. R. Gregory *et al.*, *Transplantation* 59, 655 (1995); S. O. Marx, T. Jayaraman, L. O. Go, A. R. Marks, *Circ. Res.* 76, 412 (1995).
27. S. Biro, Y. M. Fu, Z. X. Yu, S. E. Epstein, *Proc. Natl. Acad. Sci. U.S.A.* 90, 654 (1993).
28. M. J. Polman, S. W. Sherwood, G. H. Gibbons, *Circulation* 92, I-101 (1995).
29. J. R. Laird *et al.*, *ibid.* 93, 529 (1996).
30. M. J. Mann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92, 4502 (1995).
31. R. L. Geary *et al.*, *Hum. Gene Ther.* 5, 1211 (1994); T. Asahara *et al.*, *Circulation* 91, 2793 (1995); J. M. Isner *et al.*, *ibid.*, p. 2687.
32. A. Nathan, M. A. Nugent, E. R. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* 92, 8130 (1995); M. A. Lovich and E. R. Edelman, *Circ. Res.* 77, 1143 (1995).
33. Supported by a Pew Biomedical Scholar award, the Baxter Foundation, the American Heart Association, and National Institutes of Health (NIH) grant HL-48638 (G.H.G.) and by NIH grants HL-35610, HL-35252, and HL-42663 (V.J.D.).

AAAS–Newcomb Cleveland Prize

To Be Awarded for a Report, Research Article, or an Article Published in *Science*

The AAAS–Newcomb Cleveland Prize is awarded to the author of an outstanding paper published in *Science*. The value of the prize is $5000; the winner also receives a bronze medal. The current competition period began with the 2 June 1995 issue and ends with the issue of 31 May 1996.

Reports, Research Articles, and Articles that include original research data, theories, or syntheses and are fundamental contributions to basic knowledge or technical achievements of far-reaching consequence are eligible for consideration for the prize. The paper must be a first-time publication of the author's own work. Reference to pertinent earlier work by the author may be included to give perspective.

Throughout the competition period, readers are invited to nominate papers appearing in the Reports, Research Articles, or Articles sections. Nominations must be typed, and the following information provided: the title of the paper, issue in which it was published, author's name, and a brief statement of justification for nomination. Nominations should be submitted to the AAAS–Newcomb Cleveland Prize, AAAS, Room 1044, 1200 New York Avenue, NW, Washington, DC 20005, and **must be received on or before 30 June 1996**. Final selection will rest with a panel of distinguished scientists appointed by the editor-in-chief of *Science*.

The award will be presented at the 1997 AAAS annual meeting. In cases of multiple authorship, the prize will be divided equally between or among the authors.